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Bioactive and Other Piperidine Alkaloids from *Cassia leptophylla*

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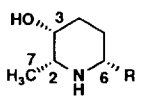
Abstract: Four new piperidine alkaloids, leptophyllin A (**4**), 3-acetylleptophyllin A (**5**), leptophyllin B (**8**), and (-)-spectaline (**1**), and 3 known piperidine alkaloids, (-)-spectalinine (**2**), canavaline (**3**), and iso-6-canavaline (**7**), were isolated by a bioassay-guided fractionation of a leaf extract of *Cassia leptophylla*. The alkaloids **1** - **3** were active in a mechanism-based DNA-modifying yeast assay and **2** was moderately active in Vero monkey and Chinese hamster ovary cell cytotoxicity assays.

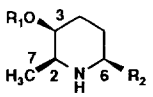
Although piperidine alkaloids are abundant in nature and many of them are known to exhibit some biological activity,[1] to the best of our knowledge none have been reported to show any effect on DNA. In our search for potential anticancer agents employing a mechanism-based yeast bioassay for DNA-modifying agents,[2,3] we have isolated (-)-spectaline (**1**), the enantiomer of the known (+)-spectaline (**6**), three known piperidine alkaloids, (-)-spectalinine (**2**), canavaline (**3**), and iso-6-canavaline (**7**) and three new piperidine alkaloids named leptophyllin A (**4**), 3-acetylleptophyllin A (**5**), and leptophyllin B (**8**), by a bioassay-guided fractionation of a bioactive extract of the Brazilian legume, *Cassia leptophylla*. Of these, (-)-spectaline (**1**), (-)-spectalinine (**2**) and canavaline (**3**) were found to be active in our bioassay. The alkaloids **1** and **2** also showed cytotoxicity in the Vero monkey and Chinese hamster ovary cell cytotoxicity assays. In this paper we report the structure elucidation, the assignment of ^{1}H and ^{13}C NMR spectral data by application of 2D techniques, and the biological evaluation of these seven piperidine alkaloids.

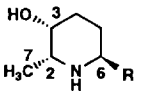
RESULTS AND DISCUSSION

A preliminary investigation of the bioactive $CHCl_3$/MeOH (2:1) extract of the leaves of *C. leptophylla* indicated that the bioactivity was due to its alkaloidal constituents. Thus the alkaloidal fraction obtained in the usual manner was subjected to CC on Al_2O_3 and the resulting fractions exhibiting bioactivity were further separated by combined Al_2O_3 CC, Si gel prep TLC, and RP-TLC as necessary to afford seven alkaloids, the structures of which were elucidated as described below. All seven alkaloids exhibited some common features in their MS, ^{1}H and ^{13}C NMR spectra (see Tables 1, 2, and 3, respectively). The presence of significant peaks at *m/z* 114 and 96 in their FABMS were characteristic of a piperidine bearing methyl, hydroxyl and alkyl functionalities.[4-6] These groups in the piperidine ring were located at C-2, C-3 and C-6, respectively, based on their ^{1}H and ^{13}C NMR data.

The least polar alkaloid, $C_{20}H_{39}NO_2$ (HRFABMS), $[\alpha]_D$ - 8.2°, in addition to the general characteristics indicated above, had a peak at *m/z* 265 in its MS which may be assigned to the fragment e (Table 1) suggesting that it has a C_{14} alkyl side chain with an oxo function, the presence of which was confirmed by its IR (ν_{max} 1720 cm^{-1}), and ^{13}C NMR (δ 209.3 s) spectra. The ^{13}C NMR spectrum, interpreted with the aid of the DEPT spectrum, indicated the presence of one quaternary C, three CH's, fourteen CH_2's, and two CH_3's. One of the CH_3 carbons appeared at δ 29.8 suggesting that the molecule contained a $COCH_3$ group. From the foregoing the structure **1** was inferred for this alkaloid. The 2D NMR data (DQCOSY, HETCOR and HMBC) supported the structure **1** proposed for this alkaloid and enabled assignment of the ^1H and ^{13}C NMR signals due to the piperidine ring and some fragments of the long alkyl chain. Thus in the HETCOR spectrum, the methine signal at δ_C 55.7 (C-2) correlated with the dq at δ_H 2.72, whilst the methine signal at δ_C 57.2 (C-6) correlated with the multiplet at δ_H 2.50; the methyl signal at δ_C 29.8 showed a correlation with the signal at δ_H 2.16. The HMBC spectrum showed ^1H-^{13}C correlations (Fig. 1) which were useful in the assignment of ^1H and ^{13}C NMR signals of the piperidine nucleus, the C-2-Me, and some portions of the side chain. The ^1H and ^{13}C NMR assignments for **1** are presented in Tables 2 and 3, respectively.



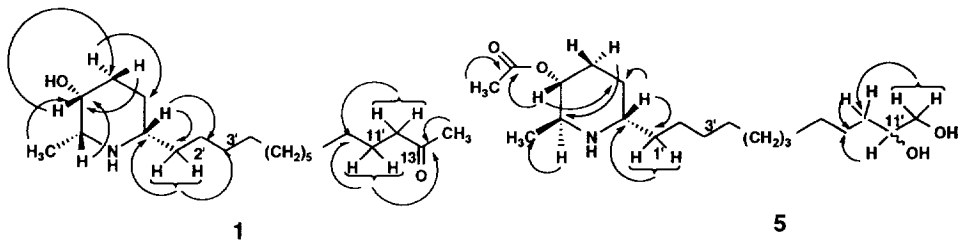




1 R = -$(CH_2)_{12}COCH_3$ **4** R_1 = H ; R_2 = -$(CH_2)_{10}CH(OH)CH_2OH$ **7** R = -$(CH_2)_{10}CH(OH)CH_3$

2 R = -$(CH_2)_{12}CH(OH)CH_3$ **5** R_1 = Ac ; R_2 = -$(CH_2)_{10}CH(OH)CH_2OH$ **8** R = -$(CH_2)_{10}CO_2H$

3 R = -$(CH_2)_{10}CH(OH)CH_3$ **6** R_1 = H ; R_2 = -$(CH_2)_{10}COCH_3$

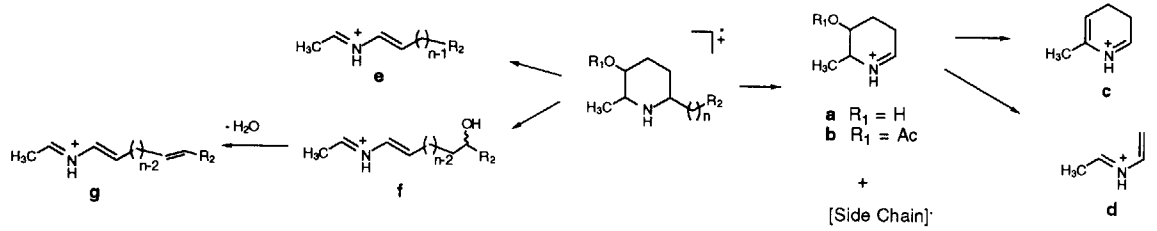


1 **5**

Fig. 1. Some selected HMBC correlations of 1 and 5.

The relative configurations of the chiral carbons C-2, C-3, and C-6 in the piperidine ring of **1** were assigned mainly on the basis of ^1H coupling data obtained from its ^1H and DQCOSY NMR spectra and comparison of these data with those reported for related piperidines.[7] It is known that in the absence of any bulky substituents in the piperidine ring, the C-3-OH group prefers the axial orientation which allows it to form an intramolecular H-bond with the lone-pair of electrons on N.[8,9] This was reflected in the IR spectrum of **1** in CCl_4 which showed a large H-bonded OH stretch signal at 3350 cm^{-1}, similar to those shown by cassine[10] and spectaline (**8**).[9] The ^1H and ^{13}C NMR data for **1** were almost identical with those reported for spectaline.[9] However, the $[\alpha]_D$ for **1** was determined to be -8.2° whereas that reported for spectaline is +8.0°, indicating that alkaloid **1** is the enantiomer of spectaline, i.e. (-)-spectaline. Comparison of the ^{13}C NMR data for **1** with those of (+)-spectaline (**8**)[9] showed a discrepancy in the literature assignments for C-2, C-6, and C-14 and these have now been revised based on HETCOR and HMBC data (see Table 2).

Table 1. MS Data for Alkaloids **1** - **5**, **7**, and **8**.



Alkaloid	M+1 (%) R^1 = H	R^1 = Ac	M-H_2O (%)	a (%)	b (%)	c (%)	d (%)	e (%)	f (%)	g (%)	Side chain (%)
1	326 (80)	-	-	114 (50)	-	97 (20)	76 (15)	265 (5)	-	-	210 (10), 198 (5)
2	328 (60)	-	311 (22)	114 (42)	-	97 (16)	-	-	267 (4)	249 (5)	214 (5), 154 (6)
3	300 (30)	-	282 (5)	114 (100)	-	96 (14)	-	-	253 (10)	235 (5)	-
4	316 (100)	-	-	114 (23)	-	96 (23)	-	255 (5)	-	237 (4)	154 (85)
5	-	358 (63)	340 (5)	-	156 (90)	97 (47)	76 (65)	255 (5)	-	237 (4)	202 (10)
7	300 (20)	-	282 (10)	114 (40)	-	96 (17)	-	-	253 (5)	-	154 (56)
8	300 (100)	-	282 (15)	114 (36)	-	96 (29)	-	253 (4)	-	-	176 (15), 154 (90)

Table 2. ^{1}H NMR (400 MHz) Spectral Data for Alkaloids **1** - **5**, **7**, and **8**.[a,b]

Proton	**1**[c]	**2**[c]	**3**[c]	**4**[c]	**5**[c]	**7**[c]	**8**[d]
2	2.72 dq (6.5,1.2)	2.72 dq (6.5,1.2)	2.72 dq (6.5,1.2)	2.72 dq (6.5,1.2)	2.81 dq (6.5,1.2)	3.21 dd (6.5,2.6)	3.28 dd (6.8,2.8)
3	3.57 br s	3.57 br s	3.57 br s	3.53 br s	4.80 br s	3.81 br s	3.82 br s
4a	1.84 ddd (13,6,6)	1.89 ddd (12.3,6,6)	1.89 ddd (12,6,6)	1.85 ddd (13,6,6)	1.98 ddd (12.5,6,6.5)	1.95 ddd (13,4,6.6)	1.95 m
4b	1.45 m	1.50 m	1.55 m	1.55 dt (13,6)	1.60 dddt (12.5,6.5,3)	1.70 dd (4,6.6)	
5a	1.40 m	1.51 m	1.50 m	-	-	1.76 m	1.42 m
5b	-	1.32 m	1.32 m	1.33 m	-		
6	2.50 m	2.48 m	2.48 m	2.51 m	2.48 m	3.02 m	3.02 m
7	1.06 d (6.5)	1.06 d (6.5)	1.05 d (6.5)	1.08 d (6.5)	1.05 d (6.5)	1.30 d (6.5)	1.31 dt (6.8)
1'	1.32 m	1.35 m	1.35 m	1.35 m	1.39 m	2.33 dt (7.0)	2.14 dt (5.9,6.9)
2'-10'	1.22 br s	1.12 br s	1.12 br s	1.20 br s	1.20 br s	1.20 br s	1.22 m
11'	1.50 dt (7.4,6)	1.25 m	3.75 m	3.69 m	3.88 m	3.45 m	2.46 t (9)
12'	2.36 t (7.4)	1.45 m	1.15 d (6.5)	H_a 3.40 dd (11,8)	H_a 3.63 dd (10,3)	1.30 d (7.6)	-
				H_b 3.62 dd (11,3)	H_b 3.41 dd (10,7.6)		
13'	-	3.75 m	-	-	-	-	-
14'	2.16 s	1.14 d (6.1)	-	-	-	-	-

[a] Chemical shifts in ppm relative to internal TMS; coupling constants (Hz) in parenthesis.
[b] Assignments based on DQCOSY (^{1}H-^{1}H COSY) and HETCOR spectra.
[c] In $CDCl_3$. [d] In CD_3OD.

Alkaloid **2**, $C_{20}H_{41}NO_2$ (HRFABMS), $[\alpha]_D$ - 8.6°, whilst having the general spectral characteristics of a 2,3,6-trisubstituted piperidine alkaloid, showed peaks at *m/z* 267 and 249 in its MS due to the fragments f and g respectively (Table 1), suggesting the presence of a hydroxy group in the side chain. The ^1H NMR spectrum was found to be similar to that reported for (-)-spectalinine.[11] As ^{13}C NMR assignments for spectalinine have not been reported previously, spectral assignment was undertaken with the aid of HETCOR, DEPT, HMBC and COLOC techniques. ^{13}C NMR assignments for (-)-spectalinine (**2**) are given in Table 3.

Table 3. ^{13}C NMR (100 MHz) Spectral Data of Piperidine Alkaloids **1-5, 7,** and **8**.[a,b]

Carbon	**1**[c]	**2**[c]	**3**[c]	**4**[c]	**5**[c]	**7**[c]	**8**[d]
2	55.7 d	55.7 d	55.7 d	55.8 d	53.9 d	58.5 d	58.5 d
3	67.9 d	68.0 d	68.0 d	68.0 d	70.5 d	66.0 d	66.0 d
4	32.0 t	32.0 t	32.0 t	32.0 t	31.9 t	31.1 t	31.1 t
5	26.1 t	26.1 t	26.1 t	26.3 t	26.6 t	25.7 t	25.7 t
6	57.2 d	57.2 d	57.1 t	57.2d	56.6 d	57.3 d	57.5 d
7	18.7 q	18.7 q	18.7 t	18.6 q	18.4 q	15.9 q	15.9 q
1'	37.1 t	37.0 t	37.1 t	36.9 t	36.8 t	36.4 t	34.4 t
2'	25.9 t	25.8 t	25.8 t	25.5 t	25.9 t	27.4 t	27.6 t
3'	29.2 t	29.5 t[e]	29.6 t[e]	29.8 t	29.2 t	29.9 t	30.7 t
4'	29.1 t[e]	29.5 t[e]	29.2 t[e]	29.8 t[e]	29.4 t[e]	29.9 t[e]	30.6 t[e]
5'	29.3 t[e]	29.6 t[e]	29.3 t[e]	29.7 t[e]	29.5 t[e]	29.8 t [e]	30.5 t[e]
6'	29.4 t[e]	29.6 t[e]	29.3 t[e]	29.7 t[e]	29.6 t[e]	29.5 t[e]	30.1 t[e]
7'	29.4 t[e]	29.6 t[e]	29.4 t[e]	29.6 t[e]	29.7 t[e]	29.4 t[e]	30.1 t[e]
8'	29.4 t[e]	29.8 t[e]	29.4 t	29.6 t	29.7 t[e]	30.0 t	29.9 t[e]
9'	29.5 t[e]	29.8 t	25.7 t	23.1 t	22.7 t	23.6 t	30.0 t
10'	29.9 t	29.8 t	38.6 t	33.2 t	33.1 t	38.8 t	23.6 t
11'	23.8 t	25.7 t	68.0 d	72.2 d	72.7 d	68.9 d	40.0 t
12'	43.9 t	39.3 t	23.4 q	66.8 t	66.8 t	23.6 q	182.8 s
13'	209.3 s	68.1 d	-	-	-	-	-
14'	29.8 q	23.4 q	-	-	-	-	-

[a]Multiplicities determined by DEPT experiments.

[b]Assignments based on HETCOR and HMBC experiments.

[c]In $CDCl_3$. [d]In CD_3OD.

[e]Values in the same column may be interchanged.

Spectral data (MS, ^1H and ^{13}C NMR) of the next most polar alkaloid, $C_{18}H_{37}NO_2$ (HRFABMS), closely resembled those of alkaloid **2** except that they indicated that it had two fewer CH_2 units in the side chain compared with **2** [MS (Table 1) and ^{13}C NMR (Table 3) evidence]. The comparison of ^1H NMR (Table 2), MS data and $[\alpha]_D$ of this alkaloid with canavaline (**3**)[4] confirmed its identity.

Spectral data for alkaloid **4**, $C_{13}H_{38}NO_3$ (HRFABMS), $[\alpha]_D$ + 2.5°, indicated it to be new and it was therefore named leptophyllin A. The presence of a 2-methyl-3-hydroxy-6-alkylpiperidine nucleus was apparent from its MS fragmentation (Table 1) and ^1H NMR data (Table 2). The MS, in addition to the general fragments depicted in Table 1, showed two prominent peaks at *m/z* 284 and 254 corresponding, respectively, to the loss of CH_2OH and $CH(OH)CH_2OH$ fragments from M^+. The ^1H NMR spectra, analyzed with the help of its DQCOSY spectrum, indicated the presence of two spin systems in addition to those due to the substituted piperidine ring, appearing at δ 3.69 m, 3.62 dd, and 3.40 dd and attributable to the side chain. Furthermore, the ^{13}C NMR spectrum, analyzed with the aid of DEPT and HETCOR spectra, showed the presence of oxygenated methine (δ_C 72.2) and methylene (δ_C 66.8) carbons. The foregoing suggested the presence of a 1,2-glycol system at the terminus of the side chain. The presence of this system as well as the

2-methyl-3-hydroxy-6-alkylpiperidine structural moiety was confirmed by the HMBC spectrum. The HMBC correlations observed for the side chain of **4** were similar to those observed for **5** (Fig. 1). Based on the above observations, along with analysis of its ^{13}C NMR spectrum, leptophyllin A was identified as 2(*S*)-methyl 6(*R*)-(11',12'-dihydroxydodecyl)piperidin-3(*S*)-ol (**4**). The stereochemical dispositions of the groups at C-2, C-3, and C-6 in **4** were deduced from the corresponding ^1H NMR coupling constants.[8,9] However, it was not possible to assign the configuration of C(11)-OH due to the limited quantity of **4** available.

A minor alkaloid was also isolated from the crude CC fraction which contained **4**. This alkaloid had ^1H and ^{13}C NMR spectral characteristics similar to **4** except that it contained an acetyl function [δ_H 2.09 s (3H); δ_C 171.0 (s) and 21.3 (q)] and appeared to be a monoacetyl derivative of **4**. The downfield shifts observed for H-3 and C-3 in its ^1H and ^{13}C NMR spectra respectively indicated it to be 3-acetylleptophyllin A (**5**). This structure was fully corroborated by MS, ^1H NMR, ^{13}C NMR and HMBC spectra. Some important HMBC correlations observed are depicted in Fig. 1.

The physical data of the next most polar alkaloid, $C_{17}H_{35}NO_2$, agreed well with those reported for iso-6-canavaline (**7**).[11] The most polar alkaloid, $C_{14}H_{33}NO_3$ (HRFABMS), $[\alpha]_D$ -3.3°, named leptophyllin B, had a singlet at δ_C 182.8 in its ^{13}C NMR spectrum, indicating the presence of a CO_2H group. Evidence for the attachment of CO_2H to the terminal carbon of the side chain was obtained by analysis of its ^1H NMR spectrum, which had a triplet due to 2H at δ_H 2.46 (Table 2). The MS, ^1H and ^{13}C NMR spectral data (Tables 1, 2, and 3, respectively) were compatible with the structure **8** proposed for this alkaloid.

The biological activity data for the alkaloids **1**-**3**, **7**, and **8** in our mechanism-based yeast mutant bioassays[2,3] are summarized in Table 4. Alkaloids **1** - **3** showed selective activities in RS 322YK and RS 321N assays compared with the wild-type RS 188N strain. Cytotoxic activities of **1** and **2** were evaluated in two cell lines (Table 4). (-)-Spectalinine (**2**) was found to be moderately active in both cytotoxicity assays.

Table 4. Bioactivity of *Cassia leptophylla* Alkaloids and the Standard.[a]

Compound	Organism or cell line				
	RS 322YK (rad52Y)	RS 321N	RS 188N (rad+)	Vero monkey cell line	Chinese hamster ovary cell line
1	15	17.5	126	>20	15
2	16	27	>50	9.9	9.7
3	16.5	26	>50	NT[b]	NT[b]
7	>500	>500	123	NT[b]	NT[b]
8	>200	157	126	NT[b]	NT[b]
Camptothecin	0.6	-	100	0.019	

[a]Results are expressed as IC_{12} (RS 322YK, RS 321N, RS 188N) (μg/ml) or IC_{50} (Vero monkey and Chinese hamster ovary cell lines) (μM) values.

[b]Not tested.

EXPERIMENTAL

General Experimental Procedures. These are identical to those reported in ref. 3; the ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, with TMS as internal standard; DQCOSY, DEPT, HETCOR, COLOC, and HMBC NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences; IR spectra were obtained on a Perkin-Elmer 727B spectrophotometer; for CC, Al_2O_3 (neutral) grade III was used; TLC and PTLC involved neutral Al_2O_3, Si gel and Whatman $LKC_{18}F$ plates; visualization on TLC was by spraying with Dragendorff and iodoplatinate reagents.

Plant Material. The plant material was collected in Sao Paulo State, Brazil, in Jan. 1993, and identified by Dr. Claudia M. Young. A voucher specimen has been deposited in the herbarium of the Botanic Gardens of Sao Paulo State, Brazil.

Bioassays. Identical to those reported in ref. 3.

Extraction and Isolation of Alkaloids. The semi-dried leaves of *C. leptophylla* Vog. (3.5 kg) were extracted with cold $CHCl_3$-MeOH (2:1), concentrated, redissolved in $CHCl_3$ and the $CHCl_3$ soluble fraction was extracted (x 3) with 2N aqueous HCl. The aqueous extracts were pooled, basified (pH = 9) with NH_4OH, and successively extracted with $CHCl_3$ and EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated to yield the crude alkaloidal mixture (256 mg). This was subjected to CC on Al_2O_3; elution with mixtures of CH_2Cl_2-MeOH of increasing polarity afforded 4 crude fractions containing alkaloids. Fraction 1 was further separated by CC on Al_2O_3 (solvent gradient, 1 - 10% EtOAc in CH_2Cl_2) followed by RP-TLC (20% H_2O in MeOH) to furnish **1** (5.0 mg) and **2** (8.0 mg). Fraction 2 was submitted to Si gel PTLC (5% MeOH in CH_2Cl_2, triple elution) to afford **3** (2.8 mg). From fraction 3 were isolated **4** (1.8 mg), **5** (4.8 mg), and **7** (9.8 mg) by CC on Al_2O_3 (solvent gradient, 1 - 10% EtOAc in CH_2Cl_2). Purification of fraction 4 by RP-TLC (10% H_2O in MeOH) afforded pure **8** (2.8 mg).

(-)-Spectaline (1). White amorphous solid, mp 118°; $[\alpha]_D$-8.2° (c = 0.32, $CHCl_3$); IR (KBr): 3350, 2860, 1720, 1560, 1370, 720 cm^{-1}; HRFABMS, *m/z* (rel. int. %): 326.3073 $[M+1]^+$(100) (calc. for $C_{20}H_{40}NO_2$: 326.3059) for remaining MS data, see Table 1; ^1H NMR (see Table 2); ^{13}C NMR (see Table 3).

(-)-Spectalinine (2). Colorless oil; $[\alpha]_D$-8.5° (c = 0.03, $CHCl_3$) (lit. -8.5°); IR (neat): 3450, 2915, 2860, 1570, 1380, 720 cm^{-1}; HRFABMS, *m/z* (rel. int. %): 328.3201 $[M+1]^+$(100) (calc. for $C_{20}H_{42}NO_2$: 328.3215), for remaining MS data, see Table 1; ^1H NMR (see Table 2); ^{13}C NMR (see Table 3).

Canavaline (3). Colorless oil; HRFABMS (see Table 1); ^1H NMR (see Table 2);^{13}C NMR (see Table 3).

Leptophyllin A (4). Colorless oil; $[\alpha]_D$ + 2.5° (c = 0.02, $CHCl_3$); HRFABMS, *m/z* (rel. int. %); 316.2847 $[M+1]^+$(100) (calc. for $C_{18}H_{38}NO_3$: 316.2851), for remaining MS data, see Table 1; ^1H NMR (see Table 2); ^{13}C NMR (see Table 3).

Acetylleptophyllin A (5). Colorless oil; $[\alpha]_D$ + 3.0° (c = 0.03, $CHCl_3$); HRFABMS, *m/zI* (rel. int. %): 358.3025 $[M+1]^+$(62) (calc. for $C_{20}H_{40}NO_4$: 358.2957), for remaining MS data, see Table 1; ^1H NMR (see Table 2); ^{13}C NMR (see Table 3).

Iso-6-canavaline (7). Colorless oil; $[\alpha]_D$ -6.0° (c = 0.05,$CHCl_3$) (lit.[11] -5.66°); HRFABMS, (see Table 1); ^1H NMR (see Table 2); ^{13}C NMR (see Table 3).

Leptophyllin B (8). Colorless oil; $[\alpha]_D$ -3.6° (c = 0.03, MeOH); HRFABMS, *m/z* (rel. int. %): 300.2526 $[M+1]^+$(100) (calc. for $C_{17}H_{34}NO_3$: 300.2538), for remaining MS data, see Table 1; ^1H NMR (see Table 2); ^{13}C NMR (see Table 3).

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